

Physicochemical Properties, Bioactive Compounds and Antioxidant Activity of Kareish Cheese Fortified with *Spirulina platensis*

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Abstract: *Spirulina* has positive effect of human health, the original chemical composition increased the nutritional content. The aim of this study was to evaluate the effects of the incorporation of *Spirulina platensis* on properties of Kareish cheese. Kareish cheese was fortified with *S. platensis* in a small grain shape at three levels: 0.5, 1 and 1.5% and a sample without *S. platensis* was made as control. Organoleptic properties, proximate composition, iron, total phenolic compounds, total flavonoids, β -carotene, DPPH, color parameters and texture profile of Kareish cheese fortified with *S. platensis* were determined. Addition of *S. platensis* to Kareish cheese resulted an increase ($P < 0.05$) in protein, fat, ash, acidity, iron, total phenols, total flavonoids, β -carotene, DPPH and enhanced the texture profile of Kareish cheese fortified with *S. platensis* when compared with control. Among the samples fortified with *S. platensis* the panelists preferred Kareish cheese samples enriched with *S. platensis* (especially 1% level). Also, addition *S. platensis* in a small grain shape not powder on cheese was preferred by panelists because make Kareish cheese like Roquefort cheese. Consequently, on the basis of these results *S. platensis* in a small grains shape enriched cheese may have a great potential for the industry to develop the shapes, texture and functional products.

Key words: Kareish Cheese • *Spirulina platensis* • Total phenolic compounds • Total flavonoids • β -carotene • DPPH

INTRODUCTION

One of the most important health problems in developing countries is that lot of people suffers from malnutrition. So, many food manufacturers used additives to raise the nutritional value of products. In the last years microalgae biomass for supplemented food applications have developed significantly [1]. Microalgae is the best products in the future because they do not need arable land and have bioactive nutrient and energy compounds which promote health and prevent from chronic disease [2]. Additives microalgae for healthy foods have increased over the last two decades [3]. In 1950, the United States and Japan began the experimental cultivation of *Spirulina* to investigate its chemical composition and possible industrial applications. In 1970 the nutritional and medicinal studies on *Spirulina* have proliferated [4], it can be a suitable source of protein for malnourished people [5]. *Spirulina* have a protein content as high as 55% to 70% of the total dry weight [6], also

have an amino acid profile that compares well with egg, notably containing all of the essential amino acids (EAA) that humans cannot synthesize and must obtain from foods [7]. *Spirulina* microalgae biomass has biological benefits such as antioxidant, anti-obesity, anti-inflammatory and anticarcinogen [8, 9]. *Spirulina* contains phenolic and flavonoids compounds [10] *Spirulina* has high pigments content which improve the antioxidant activity [11]. Also, *Spirulina* contained β -carotene which can neutralize free radical substances in human body [12] which can promote the existence of degenerative diseases [13], they found that 6 mg d⁻¹ can reduce cancer risk for human [14, 15]. Women on weight loss diets typically do not get enough iron and can become anemic, it is also essential for strong red blood cells and a healthy immune system. *Spirulina* have iron twice as absorbable as the form of iron found in vegetables [16]. *S. platensis* has probiotic efficiency of for lactic acid bacteria [17]. *S. platensis* has antifungal effect of *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida*

tropicalis, *Candida albicans* and *Candida glabrata* and has antimicrobial effect of *Bacillus subtilis*, *B. cereus* and *Escherichia coli* [18] and some human pathogens [19]. Addition of *spirulina* was considered as the best concentration for soft cheese gave significant effect to protein, water, fat, β -carotene and texture [13]. Kareish cheese is one of the important part of consumer diet and known as popular local in Egyptian cities, sometimes it consumed more than one times a day [20]. Little researches have been studied the application of *Spirulina* on cheese and all research added *Spirulina* in powder shape that cased green cheese which not acceptable for most consumers. The current study added small grains of *S. platensis* to Kareish cheese and evaluated effects on organoleptic properties, proximate composition, iron, total phenols, total flavonoids, β -carotene, DPPH, color parameters and texture profile and then determined the maximum concentration of *S. platensis* that can be added to the product, thus, will be acceptable for consumption.

MATERIALS AND METHODS

Materials: Pasteurized buffalo skim milk and salt were purchased from the local market in Assiut, Egypt. Commercial yoghurt culture containing *Streptococcus thermophiles* and *Lactobacillus delbrueckii subsp. bulgaricus* was used for manufacturing of Kareish cheese. *S. platensis* were purchased from DXN Egypt company, Cairo, Egypt.

Methods

Cheese Preparing: Kareish cheese produced according to Abou-donia [21]. Buffalo's skim milk was heated to 85°C for 15 sec and cooled to 38-40°C. Active starters of *S. thermophilus* and *L. bulgaricus* (3% w/w) stirred well and held until to coagulate. Cutting coagulates to small moulds of cheese and divided it into four portions, as follows: The first portion was kept untreated (control). The second, third and fourth portions put 0.5%, 1% and 1.5% small grains of *S. platensis*, respectively with addition (0.5%, w/v of milk) sodium chloride for all portion between cheese layers and left till whey drain into small cheese at room temperature for 4–5 h. Cheese blocks were monitored to different analyses after overnight storage at $5^{\circ} \pm 1^{\circ} \text{C}$.

Proximate Analysis: Kareish samples were analyzed for titratable acidity (T.A), fat, protein and ash contents as described in A.O.A.C. [22], iron was determined as Jackson [23], β -carotene analysis as described in Costache *et al.* [24].

Phenolic, Total Flavonoid Contents and Antioxidant Activity by Radical DPPH Scavenging Activity of Kareish Cheese:

Twenty grams of Kareish cheese were extracted using petroleum ether, tetrahydrofuran and methanol in succession using soxhlet apparatus according to the methods of Roopalatha and Nair [25] with some modifications. Each extract obtained following extraction step was filtered using filter paper Whatman No 1, dried using rotary evaporator and the yield of each extract thus obtained was recorded. Different extracts were reconstituted in 10 mL dimethylsulfoxide (DMSO) and stored under nitrogen at -30°C till further use the total phenolic content was determined according to the Folin-Ciocalteu procedure [26]. Briefly, the extract (500 μL) was transferred into a test tube and oxidized with the addition of 250 μL of Folin-Ciocalteu reagent. After 5 min, the mixture was neutralized with 1.25 ml of 20% aqueous Na_2CO_3 solution. After 40 min, the absorbance was measured at 725 nm against the solvent blank. The total phenolic content was determined by means of a calibration curve prepared with Gallic acid and expressed as μg of Gallic acid equivalent (GAE) per ml of sample. The total flavonoid content was determined according to Zilic *et al.* [26]. Briefly, 250 μL of 5% NaNO_2 was mixed with 500 μL of extract. After 6 min, 2.5 ml of a 10% AlCl_3 solution was added. After 7 min, 1.25 ml of 1 M NaOH was added and the mixture was centrifuged at 5000 g for 10 min. Absorbance of the supernatant was measured at 510 nm against the solvent blank. The total flavonoid content was expressed as μg of catechin equivalent (CE) per ml of sample. Free radical scavenging capacity was determined using the stable 1,1-Diphenyl-2-picryl-hydrazyl (DPPH $^{\bullet}$) according to Hwang *et al.* [27]. The final concentration was 50 μM for DPPH $^{\bullet}$ and the final reaction volume was 3.0 mL. The absorbance at 517 nm was measured against a blank of pure methanol at 60 min. Percent inhibition of the DPPH free radical was calculated by the following equation:

$$\text{Inhibition (\%)} = 100 \times (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}$$

where: A_{control} is the absorbance of the control reaction (containing all reagents except the test compound). A_{sample} is the absorbance of the test compound. Also, the antioxidant activity was determined by means of a calibration curve prepared with Trolox and expressed as mg of Trolox equivalent (TE) per unit (volume or weight) of sample.

Measurement of the Color of Kareish Cheese: Hunter color parameter: Changes in Hunter color parameter (L, a & b) of different blends (flours) were followed up using Tristimulus Color Analyzer (Hunter, Lab Scan XE, Reston, Virginia) with standard white tile.

Texture Profile Analysis: Texture profile analysis was performed on cheese samples using the double compression test (Multi test 1d Memesin, Food Technology Corporation, Slinfold, W. Sussex, UK). Experiments were carried out by a compression test that generated a plot of force (N) versus time (s). A 25- mm-diameter perplex conical-shaped probe was used to perform the analysis at five different points on the sample surface. In the 1st stage, the sample was compressed by 80% of their original depth at a speed of 2 cm/min during the pretest, compression and the relaxation of the sample. From the force–time curve, the following parameters were determined according to the definition given by the IDF [28];

Hardness (N) = maximum force of the 1st compression

Cohesiveness = area under the 2nd compression/ area under the 1st compression (A_2/A_1)

Adhesiveness (N.s) = negative area in the curve.

Springiness (mm) = length 2nd compression/ length 1stcompression (L_2/L_1)

Gumminess (N) = Hardness × cohesiveness

Chewiness (mm) = gumminess × springiness

Organoleptic Characteristics of Kareish Cheese: Fifteen panelists (7 males and 8 females, aged between 25 and 45 years) who have experience with white cheese and regularly used its descriptive vocabulary, were participated. The cheese samples were scored for color and appearance (15 points), flavor (50 points), body and texture (35 points) and over all acceptability (100 points) according to IDF [29]. Panel members were also instructed to report any defects or unpleasant flavor.

Statistical Analysis: Statistical analysis of experimental data was performed by analysis of variance (ANOVA) producers using IBM SPSS statistics version 21 program to examine statistical significance differences of experimental data. Results were considered statistically significant when $p < 0.05$. Mean \pm standard deviation values were also presented.

RESULTS AND DISCUSSION

Organoleptic Properties of Kareish Cheese Fortified by

S. Platensis: Data presented in Table 1 show that the organoleptic properties of Kareish cheese fortified with 0.5%, 1.0% and 1.5% small grains of *S. platensis*. The color and appearance observe difference ($p < 0.05$) among of Kareish cheese samples fortified with *S. platensis*. The concentration 1.0% *S. platensis* recorded the highest score among the Kareish cheese fortified *S. platensis* samples that may due to increasing microalgae causes darken of Kareish cheese and decreasing causes light green color. Furthermore, the manufacture Kareish cheese by using small grains of *S. platensis* was preferred by panelists because the color of Kareish cheese fortified with microalgae was like Roquefort cheese. Increasing microalgae than 1.5% causes darken of Kareish cheese and consumers rejected of primary experiment. The flavor of Kareish cheese fortified with *S. platensis* have significantly ($p < 0.05$) different among the Kareish cheese samples except between control sample and sample containing 0.5% *S. platensis* there is no significantly ($p > 0.05$) differences between them. The body and texture of Kareish cheese fortified with *S. platensis* have significantly ($p < 0.05$) different among the Kareish cheese samples. Kareish cheese fortified with 1.5% *S. platensis* recorded the highest score of body and texture that due the reduction of water and increase of dry matter by *S. platensis* [30]. Overall acceptability of Kareish cheese fortified with 1% *S. platensis* gained the highest scores than fortified with 0.5% and 1.5% *S. platensis* that due to 0.5% light green color and 1.5% dark green [31]. Generally, all Kareish cheese samples had good organoleptic properties, but the most preferable one was the Kareish cheese fortified with 1% *S. platensis*.

Proximate Analysis of Kareish Cheese Fortified by

S. Platensis: The contents of protein, fat, ash, acidity and iron contents in Kareish cheese samples are shown in Table 2. According to Parada *et al.* and Beheshtipour *et al.* [32, 33] *S. platensis* is a good source of proteins (60–70% of its dry weight). Table 2 shows that increasing in the *S. platensis* increases ($P < 0.05$) the content of protein in the Kareish cheeses. The highest content of protein was in Kareish cheese with 1.5% *S. platensis*, the increasing of protein with increasing *S. platensis* due to the high protein content in *S. platensis* [34] which ranged from 55% to 75% [35], 6% to 62% [36], 60% to 71% [3],

Table 1: Organoleptic properties of Kareish cheese samples fortified by *Spirulina* biomass (mean \pm standard deviation)

Level of (<i>S. platensis</i>)	Color & Appearance	Flavor	Body & Texture	Over all acceptability
Organoleptic scores	15	50	35	100
Control 0%	14.8 \pm 0.33 ^a	48.0 \pm 1.35 ^a	33 \pm 0.74 ^b	95.5 \pm 2.12 ^a
0.5%	13.0 \pm 0.30 ^d	48.0 \pm 2.08 ^a	32.5 \pm 0.44 ^c	82 \pm 0.91 ^c
1.0%	14.5 \pm 0.22 ^b	47.7 \pm 1.74 ^b	33 \pm 0.36 ^b	91 \pm 1.43 ^b
1.5%	13.9 \pm 0.21 ^c	47.5 \pm 0.64 ^c	33.5 \pm 0.52 ^a	79 \pm 0.69 ^d

^{a,b,c} Values in the same columns having different superscripts are significantly different (p<0.05)

Table 2: Proximate analysis of Kareish cheese samples fortified by *Spirulina* biomass (mean \pm standard deviation)

Level of (<i>S. platensis</i>)	Protein (%)	Fat (%)	Ash (%)	Acidity (%)	Iron (Mg/100gm)
Control 0%	13.34 \pm 0.05 ^c	0.24 \pm 0.01 ^c	2.41 \pm 0.05 ^d	0.98 \pm 0.07 ^c	0.93 \pm 0.02 ^d
0.5%	13.44 \pm 0.07 ^b	0.28 \pm 0.01 ^b	2.42 \pm 0.09 ^c	2.08 \pm 0.03 ^b	1.33 \pm 0.01 ^b
1.0%	12.11 \pm 0.04 ^d	0.28 \pm 0.03 ^b	2.83 \pm 0.10 ^b	1.87 \pm 0.04 ^d	1.0 \pm 0.01 ^c
1.5%	14.63 \pm 0.14 ^a	0.31 \pm 0.01 ^a	3.03 \pm 0.14 ^a	2.13 \pm 0.06 ^a	2.05 \pm 0.01 ^a

^{a,b,c} Values in the same columns having different superscripts are significantly different (p<0.05)

Table 3: Total Phenolic compounds, total Flavonoids, β -carotene and DPPH of Kareish cheese samples fortified by *S. platensis* (mean \pm standard deviation)

Level of (<i>S. platensis</i>)	Total Phenols (mg GAE/100g)	Total Flavonoids (mg CE/100g)	β -carotene (mg/100g)	DPPH (mg TE/100g)
Control 0%	14.525 \pm 0.23 ^d	3.361 \pm 0.03 ^d	0.191 \pm 0.07 ^d	4.199 \pm 0.13 ^d
0.5%	17.949 \pm 0.36 ^b	5.114 \pm 0.05 ^c	0.551 \pm 0.15 ^c	4.395 \pm 0.22 ^b
1.0%	17.257 \pm 0.33 ^c	6.250 \pm 0.12 ^b	0.609 \pm 0.10 ^b	4.219 \pm 0.09 ^c
1.5%	18.437 \pm 0.27 ^a	6.391 \pm 0.21 ^a	1.020 \pm 0.04 ^a	4.688 \pm 0.15 ^a

^{a,b,c} Values in the same columns having different superscripts are significantly different (p<0.05)

69% to 74% [37]. Other studies reported that addition of *Spirulina* has increased significantly (P<0.05) the protein content in cyanobacterial cheeses [30] and in yogurt [38]. The fat content of Kareish cheese fortified by *Spirulina* microalgae biomass (0.5%, 1% and 1.5%) has significant difference (P < 0.05) this may be due to the fat content in *Spirulina* 2.64% [37]. The Acidity of Kareish cheese fortified by *S. platensis* (0.5, 1 and 1.5%) increased (p<0.05) when *S. platensis* was added, this may be due to the fact that the *Spirulina* biomass stimulated the growth of starters [38, 39]. Bhowmik *et al.* [17] recorded that *S. platensis* enhance the growth of lactic acid bacteria. Antimicrobial effect of *S. platensis* on pathogenic bacteria and antifungal effect [18] cause enhance the growth of lactic acid bacteria. The ash content of Kareish cheese has significant difference (P < 0.05) with increasing of *Spirulina* biomass addition (P < 0.05). The increasing of ash in Kareish cheese due to relatively high ash content of *S. platensis* 10.66% to 11.56% [13]. Nutritional researches recorded that absorption iron from *S. platensis* was better than any other supplements; so, it is signifying a sufficient source for women [40]. The iron contents in Kareish cheeses fortified by *S. platensis* are given in Table 2. The iron levels were significantly increased (P<0.05) in all Kareish cheeses samples with increased *S. platensis*. The concentration 1.5% of *S. platensis* make the significant increase (P<0.05) of iron than that occurred in samples 0.5% and 0.1%.

Compounds, Total Flavonoids, β -Carotene and DPPH of Kareish Cheese Fortified by *S. Platensis*: The phenolic and flavonoid compounds show extensive benefits to human health with various beneficial pharmacological effects like acting as antioxidant [41]. Total Phenolic compounds and flavonoid content of Kareish cheese fortified by *S. platensis* showed in Table 3. The total phenolic and flavonoid contents were found to be lower 14.525 \pm 0.23 mg GAE/100g and 3.361 \pm 0.03 mg CE/100g in control Kareish cheese, respectively, while Kareish cheese contain 1.5% *S. platensis* scored 18.437 \pm 0.27 mg GAE/100g and 6.391 \pm 0.21 mg CE/100g, respectively (Table 3). The total phenolics and flavonoid contents significantly (p<0.05) increased with an increase of the content *S. platensis*. These results are in agreement with Minea *et al.*, [10] Who reported that *Spirulina* had a relatively higher provitamin A concentration [42]. The present study found that adding *S. platensis* caused significantly increasing (P<0.05) of β -carotene in Kareish cheese fortified by *S. platensis* that due to *Spirulina* has high amount of β -carotene 26.74% [13]. Other studied have used up to 5% (w/w) *Spirulina* for fortification chocolate bar to increase its β -carotene content [43]. The β -carotene content in Kareish cheese fortified by *Spirulina* biomass was 0.609, 0.552 and 1.021 mg/100g in samples containing 0.5, 1.0 and 1.5% *Spirulina* biomass, respectively. Food and Agriculture Organization reported that intake 6 mg d⁻¹ from β -carotene can reduce

Table 4: Color parameters of Kareish cheese samples fortified by *S. platensis* (mean \pm standard deviation).

Level of (<i>S. platensis</i>)	L*	a*	b*	ΔE
Control 0%	95.80 \pm 0.02 ^a	-1.02 \pm 0.01 ^a	7.33 \pm 0.02 ^c	96.08 \pm 0.33 ^a
0.5%	80.46 \pm 0.01 ^b	-6.89 \pm 0.03 ^b	7.79 \pm 0.02 ^a	81.12 \pm 0.10 ^b
1.0%	76.26 \pm 0.01 ^d	-9.88 \pm 0.02 ^c	7.71 \pm 0.01 ^b	77.28 \pm 0.18 ^c
1.5%	74.73 \pm 0.01 ^c	-10.69 \pm 0.01 ^d	5.78 \pm 0.01 ^d	75.71 \pm 0.19 ^d

^{a,b,c} Values in the same columns having different superscripts are significantly different (p<0.05)

Table 5: Texture Profile Analysis of Kareish cheese samples fortified by *S. platensis* (mean \pm standard deviation)

Level of (<i>S. platensis</i>)	Hardness N	Cohesiveness (B/A area)	Springiness mm	Gumminess N	Chewiness N/m
Control 0%	4.4 \pm 0.59 ^d	0.276 \pm 0.02 ^c	0.404 \pm 0.07 ^d	1.214 \pm 1.22 ^d	0.490 \pm 0.25 ^d
0.5%	4.5 \pm 0.51 ^c	0.326 \pm 0.04 ^b	0.462 \pm 0.05 ^a	1.467 \pm 0.41 ^b	0.677 \pm 0.08 ^b
1.0%	4.7 \pm 0.75 ^b	0.275 \pm 0.02 ^d	0.461 \pm 0.03 ^b	1.292 \pm 0.53 ^c	0.595 \pm 0.12 ^c
1.5%	4.9 \pm 0.52 ^a	0.328 \pm 0.01 ^a	0.440 \pm 0.02 ^c	1.607 \pm 0.64 ^a	0.707 \pm 0.10 ^a

^{a,b,c} Values in the same columns having different superscripts are significantly different (p<0.05)

human cancer risk [14]. Acceptable mechanism antioxidants inhibition is lipid peroxidation by free radical scavenging. Antioxidants affected on DPPH radical scavenging due to their hydrogen donating ability [44]. Table 3 showing Kareish cheese fortified by *S. platensis* had higher antioxidant activity than control Kareish cheese. The DPPH radical scavenging activity of control Kareish cheese was 4.199 (mgTE/100g) increased gradually as added 0.5, 1 and 1.5% *S. platensis* which values 4.395, 4.219 and 4.689 (mgTE/100g), respectively. The increase in content of chlorophylls may be cause the in the free radical scavenging [45].

The Color Evaluation of Kareish Cheese Fortified by *S. platensis*: Color of products effect the quality properties of them and it is very important factor for consumer's perceptions because, there is direct relation between the color and consuming acceptance of food products [15] especially dairy products [46]. For these reasons colorants products have increased in the resent years. Color properties of Kareish cheese fortified by *S. platensis* samples are shown in (Table 4). L* values, indicating brightness ranged from 74.73 to 80.46 for Kareish cheese fortified by *S. platensis*. The highest L* values was observed in control sample which decreased significantly by increasing amounts of *S. platensis*. Kareish cheese containing 1.5% *S. platensis* was greenish this may be due to the green color of *S. platensis* which change the color of Kareish cheese (Table 4). These results are in agreement with Ki Jeon [47] who studied the effect of addition Chlorella on cheese color. The effective of color due to whiteness reduction is related to *S. platensis* microalgae concentration [30]. The a* values, indicating redness it ranged from -6.89 to -10.69 in Kareish cheese fortified by *S. platensis*. The control and fortified Kareish cheese by *S. platensi* showed negative a* values

which decreased by increasing amounts *S. platensis*. The b* value which indicating yellowness, ranged from 5.78 to 7.79 in Kareish cheese fortified by *S. platensis* samples the control Kareish cheese showed the lowest b* values 7.33 and the value increased by increasing amounts of *S. platensis*. Overall, the present study showed that addition *S. platensis* to Kareish cheese samples decrease the a* and L* value and increased in b* value by increasing amount of *S. platensis*.

Texture Profile Analysis of Kareish Cheese Fortified by *S. Platensis*: The texture profile analyses of Kareish cheese fortified by *S. platensis* are observed in (Table 5). The texture profile analyses Kareish cheese fortified with *S. platensis* were significant (p<0.05) effect among all Kareish cheese which fortified by *S. platens*. Hardness, cohesiveness, gumminess and chewiness values recorded increased with increasing *S. platensis* to Kareish cheese but springiness values recorded decreased. Hardness increased from 4.4 \pm 0.59N in control Kareish cheese to 4.9 \pm 0.59N in Kareish cheese containing 1.5% *S. platens*. The hardness increased in Kareish cheese with increasing of *S. platensis* that due to reduction of water and increase of dry matter [30]. This results agreement with Ki Jeon [47] who reported that the rheological properties were significantly difference on cheese as added microalgae 0.3%, 0.5% *M. longifolia* L. In the present study texture analysis showed that Kareish cheeses fortified by of *S. platens* at 0.5%1.0 and 1.5% enhanced the development of texture characteristics.

CONCLUSION

The results reported that addition of 0.5, 1% and 1.5% *S. platensis* to Kareish cheese have significant effect of protein, β -carotene and antioxidant activity than in the

control Kareish cheese sample. Texture profile show differences ($P < 0.05$) among Kareish cheese fortified with *S. platensis* compared with the control sample. Panelists were preferred Kareish cheese samples enriched with *S. platensis* small grain (especially 1% level) because cheese was like Roquefort cheese. Consequently, on the basis of these results *S. platensis* small grains enriched cheese may have a great potential for the cheese industry to develop the shapes, texture and functional products.

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